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16. ABSTRACT

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Effect of Acute and Chronic Cd Exposure on Lindane Metabolism[1]

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This study reports the effects of acute and chronic Cd treatment on the metabolism of lindane by rats 3 days, 1, 2, 3, and 5 weeks after exposure to the heavy metal. Eighty adult male Wistar rats were randomly assigned to one of four exposure groups. One group was injected sc, three times a week throughout the study, with 0.75 mg of Cd/kg body weight. The other three groups received one sc injection of either 0, 1.04, or 3.20 mg of Cd/kg body weight. Twenty-four hours prior to sacrifice four rats from each group received a po dose of 1.83 mg of lindane (containing 2.5 μCi of [^{14}C]lindane). Results of this study indicate that Cd pretreatment causes inhibition of lindane metabolism which is of long duration and which is not appreciably relieved by chronic exposure to the metal.

Present in air, fresh water, food, cigarette smoke, and some pesticides and fertilizers, the ubiquitous heavy metal cadmium has been reported to inhibit hepatic drug-metabolizing enzymes in mammals (Unger and Clausen, 1973; Hadley *et al.*, 1974; Tsang and Furst, 1976; Yoshida *et al.*, 1976; Krasny and Holbrook, 1977; Teare *et al.*, 1977; Schnell, 1978). These reports were based on the effect of Cd treatment upon the *in vitro* enzyme activities, duration of drug-induced hypnosis, paralysis, tremor onset, hypothermia, and the hepatic level of cytochrome *P*-450. However, metabolic effects of Cd in the live animal must be distinguished from any changes in receptor sensitivity (Johnston *et al.*, 1975) and this can best be accomplished by investigating the alterations in storage, metabolism, and excretion of a xenobiotic after exposure to Cd. Other studies have indicated that the inhibitory effect of this heavy metal was decreased by increasing the elapsed time between Cd exposure and the assay involved (Hadley *et al.*, 1974; Schnell *et al.*, 1974; Yoshida *et al.*, 1976; Krasny and Holbrook, 1977; Teare *et al.*, 1976) and by preexposure to the metal itself (Roberts *et al.*, 1976; Yoshida *et al.*, 1976; Schnell, 1978). The present investigation was designed to study the effect of acute and chronic Cd pretreatment on the *in vivo* and *in vitro* metabolism of the organo-

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chlorine insecticide, lindane (γ-hexachlorocyclohexane, γ-HCH) at 3 days, 1, 2, 3, and 5 weeks after initial exposure.

METHODS

Eighty adult male Wistar rats, individually housed, were randomly assigned to one of four treatment groups. One group was injected sc, three times a week throughout the experiment with cadmium chloride[3] in sterile saline at 0.75 mg of Cd/kg body weight. The other three groups received one sc injection of either 0, 1.04, or 3.20 mg of Cd/kg body weight.

The dose levels were chosen to provide a subthreshold chronic dose, 0.75 mg of Cd/kg (Schnell *et al.*, 1974), an acute dose of 1.04 mg of Cd/kg at which the incorporation rate of Cd into metallothionein is linear (Cempel and Webb, 1976), and an acute dose of 3.20 mg of Cd/kg at which complete saturation occurs (Cempel and Webb, 1976). Six rats receiving 3.20 mg of Cd/kg died during the study. One died within 2 weeks, another within 3 weeks, and the remaining four within 5 weeks after this treatment was administered. The surviving rats from all treatment groups showed no overt signs of toxicity and there were no significant differences in body weight at sacrifice.

Four animals from each treatment group were sacrificed at 3 days, 1, 2, 3, and 5 weeks after the initial Cd injection. Twenty-four hours prior to sacrifice, the rats received a po dose of 1.83 mg of lindane[4] (containing 2.5 μCi of [^{14}C]lindane[5]). Individual fat, feces, and urine samples were analyzed for radioactivity. Feces samples were subjected to combustion in a Packard Tri-Carb Model 306 sample oxidizer while fat and urine were extracted as previously described (Chadwick *et al.*, 1975a) and the radioactivity was determined in a Packard Tri-Carb Model 3380 liquid scintillation spectrometer.

The Cd content of liver and kidney tissue was determined with a Perkin–Elmer Model 403 atomic absorption spectrometer as previously described (Faeder *et al.*, 1977).

Excretion of free and conjugated chlorophenols and the neutral metabolites of lindane was determined on a Tracor Model MT-220 gas chromatograph equipped with an electron capture detector and a Coulson electrolytic conductivity detector. The columns used consisted of 183 × 0.63-cm o.d. glass U-tubes packed with either 1.5% OV-17 + 1.95% QF-1 on 80/100-mesh Gas Chrom Q[6] or 5% DEGS[7] on 60/80-mesh Gas Chrom Q.[8] Both columns were maintained isothermally at 170°C and the helium carrier gas was regulated at 60 cm^3/min while hydrogen flow was 11 cm^3/min. Urinary collection and extraction procedures have been described previously (Chadwick *et al.*, 1977a).

[3] Ultrapure cadmium chloride was obtained from Ventron Corporation, Alfa Products, Danvers, Massachusetts.

[4] Lindane, 99.9% γ-hexachlorocyclohexane was obtained from the Health Effects Research Laboratory Analytical Reference Standards Repository, Research Triangle Park, N.C.

[5] The [^{14}C]lindane, uniformly labeled with a specific activity of 48 mCi/mmol and a radiochemical purity of 99% was obtained from Amersham/Searle, Arlington Heights, Ill.

[6] OV-17 (1.5%) + 1.95% QF-1 on 80/100-mesh Gas Chrom Q was obtained from Applied Sciences Laboratories, Inc., State College, Pa.

[7] Diethyleneglycolsuccinate was obtained from Supelco, Inc., Bellefonte, Pa.

[8] Gas Chrom Q (60/80 mesh) was obtained from Applied Sciences Laboratories, Inc., State College, Pa.

Hepatic content of microsomal protein (Lowry *et al.*, 1951) cytochrome *p*-450 (Omura and Sato, 1964a,b), and the enzyme activity involved in the dehydrogenation and dechlorination of lindane (Chadwick *et al.*, 1975b) were determined. Also the hydroxylation of 1,3,5- and 1,2,4-trichlorobenzene together with 1,2,3,4- and 1,2,3,5-tetrachlorobenzene to the corresponding chlorophenols was studied. For this assay the tissue prepartion, incubation conditions, and extraction procedures previously described (Chadwick *et al.*, 1975b) were used with the exceptions that a mixture containing 400 μg of each of the four chlorobenzenes in 0.1 ml of acetone was added as the substrate and the Tris–HCl buffer employed contained 2 g of $MgCl_2$/liter.

The Student's *t* test, correlation coefficients (Snedecor, 1956), and Duncan's Multiple Range Test (Duncan, 1955) were used as aids in the interpretation of the data from the study. Comparisons were considered significantly different at $P < 0.05$ unless otherwise stated.

RESULTS

Significant Cd-induced inhibition of the hydroxylation of 1,3,5- and 1,2,4-trichlorobenzene and 1,2,3,5- and 1,2,3,4-tetrachlorobenzene was observed 3 days after treatment began (Table 1). Only the pathway from 1,2,4-trichlorobenzene to 2,4,5-trichlorophenol was not significantly inhibited. One week after treatment was initiated, however, no significant inhibition of hydroxylation was noted. This agreed with results from other labs which indicated that peak Cd inhibition of hepatic drug-metabolizing enzymes occurred from 2 to 5 days after Cd administration (Hadley *et al.*, 1974; Schnell *et al.*, 1974; Yoshida *et al.*, 1976). On the other hand, the renewal of significant inhibition at later time periods, 2–5 weeks after Cd administration has not previously been reported. Similarly, at 1 and 2 weeks after treatment, the rats appeared to be recovering somewhat from the Cd-induced inhibition of lindane dehydrogenase, lindane dechlorinase, and hepatic cytochrome *P*-450 content when significant inhibition of these enzymes recurred 3 and 5 weeks after treatment (Table 2). Repeated treatment of the rats with 0.75 mg of Cd/kg only provided partial protection from the Cd-induced enzyme inhibition (Tables 1 and 2).

The effect of Cd on the storage, metabolism, an excretion of another environmental toxicant, the organochlorine insecticide lindane, is presented in Tables 3 and 4. Corresponding to the effect of Cd on *in vitro* enzyme activity (Tables 1 and 2), there was a significant Cd-induced decrease in the excretion of radiolabeled products at 3 days, followed by an apparent recovery to control levels at 1 and 2 weeks and then the recurrence of significantly reduced levels of excreted radioactivity 3 and 5 weeks after treatment began. Five weeks after treatment began, the marked Cd-induced inhibition of excreted radioactivity was comparable to that observed after 3 days. Radioactivity in the adipose tissue of the rats dosed with 1.04 and 3.20 mg of Cd/kg was significantly elevated over that in control rats at 3 days and 1 week after treatment. Only the rats dosed with 3.20 mg of Cd/kg had significantly higher levels of radioactivity in the fat than controls 2 weeks after treatment and no significant differences in stored radioactivity were observed 3 or 5 weeks after treatment with Cd.

Treatment with Cd significantly altered the distribution of radiolabelled metabolites in the urine (Table 4). Three days after treatment began, all rats exposed to Cd excreted a

TABLE 1

EFFECT OF CADMIUM ON *IN VITRO* ENZYME ACTIVITY[a]

Time after initial exposure (days)	Cd treatment[b] (mg/kg)	Hydroxylation of chlorobenzenes to chlorophenols					Treatment comparisons[h]
		I. 2,4,6-TCP[c] (μg/mg prot/30 min)	II. 2,3,5-TCP[d] (μg/mg prot/30 min)	III. 2,4,5-TCP[e] (μg/mg prot/30 min)	IV. 2,3,4,6-TTCP[f] (μg/mg prot/30 min)	V. 2,3,4,5-TCCP[g] (μg/mg prot/30 min)	
3	1. 0.00	0.67 ± 0.28	0.055 ± 0.022	0.065 ± 0.022	1.90 ± 0.86	1.37 ± 0.51	I. 1 > 4,3
	2. 1.04	0.17 ± 0.13	0.009 ± 0.007	0.034 ± 0.020	0.32 ± 0.26	0.42 ± 0.32	II. 1 > All
	3. 3.20	0.07 ± 0.03	0.001 ± 0.0001	0.015 ± 0.046	0.07 ± 0.02	0.12 ± 0.02	III. N.S.
	4. 0.75	0.12 ± 0.04	0.005 ± 0.002	0.041 ± 0.010	0.21 ± 0.04	0.24 ± 0.04	IV. 1 > All
							V. 1 > 4,3
7	1. 0.00	0.40 ± 0.17	0.025 ± 0.010	0.055 ± 0.014	0.64 ± 0.37	0.55 ± 0.13	I. N.S.
	2. 1.04	0.16 ± 0.02	0.011 ± 0.002	0.028 ± 0.004	0.26 ± 0.05	0.29 ± 0.04	II. N.S.
	3. 3.20	0.15 ± 0.04	0.010 ± 0.002	0.019 ± 0.007	0.23 ± 0.05	0.32 ± 0.10	III. N.S.
	4. 0.75	0.27 ± 0.09	0.023 ± 0.009	0.044 ± 0.018	0.38 ± 0.07	0.58 ± 0.23	IV. N.S.
							V. N.S.
14	1. 0.00	0.45 ± 0.13	0.028 ± 0.006	0.060 ± 0.008	0.87 ± 0.31	0.76 ± 0.20	I. 1 > All
	2. 1.04	0.12 ± 0.01	0.008 ± 0.001	0.020 ± 0.006	0.18 ± 0.04	0.20 ± 0.04	II. 1 > All
	3. 3.20	0.10 ± 0.01	0.005 ± 0.001	0.015 ± 0.002	0.13 ± 0.03	0.18 ± 0.03	III. 1 > All
	4. 0.75	0.14 ± 0.04	0.007 ± 0.002	0.023 ± 0.003	0.27 ± 0.12	0.20 ± 0.05	IV. 1 > 2,3
							V. 1 > All
21	1. 0.00	0.58 ± 0.02	0.038 ± 0.002	0.093 ± 0.012	1.01 ± 0.07	0.85 ± 0.12	I. 1 > All
	2. 1.04	0.11 ± 0.01	0.008 ± 0.001	0.020 ± 0.004	0.15 ± 0.03	0.19 ± 0.03	II. 1 > All
	3. 3.20	0.10 ± 0.02	0.010 ± 0.002	0.015 ± 0.002	0.15 ± 0.04	0.14 ± 0.02	III. 1 > All;
	4. 0.75	0.26 ± 0.10	0.013 ± 0.005	0.035 ± 0.008	0.44 ± 0.21	0.30 ± 0.11	4 > 2,3
							IV. 1 > All
							V. 1 > All
35	1. 0.00	0.35 ± 0.11	0.020 ± 0.005	0.044 ± 0.008	0.70 ± 0.20	0.50 ± 0.11	I. 1 > 2
	2. 1.04	0.11 ± 0.02	0.010 ± 0.004	0.059 ± 0.042	0.29 ± 0.11	0.16 ± 0.02	II. N.S.
	4. 0.75	0.19 ± 0.04	0.010 ± 0.002	0.032 ± 0.005	0.39 ± 0.10	0.30 ± 0.07	III. N.S.
							IV. N.S.
							V. 1 > 2

[a] Values are means ± SE.
[b] Treatment described under Methods.
[c] *In vitro* metabolism of 1,3,5-trichlorobenzene to 2,4,6-trichlorophenol.
[d] *In vitro* metabolism of 1,2,4-trichlorobenzene to 2,3,5-trichlorophenol.
[e] *In vitro* metabolism of 1,2,4-trichlorobenzene to 2,4,5-trichlorophenol.
[f] *In vitro* metabolism of 1,2,3,5-tetrachlorobenzene to 2,3,4,6-tetrachlorophenol.
[g] *In vitro* metabolism of 1,2,3,4-tetrachlorobenzene to 2,3,4,5-tetrachlorophenol.
[h] Comparisons are significantly different at $P < 0.05$ by Duncan's Multiple Range Test.

TABLE 2

EFFECT OF CADMIUM ON *IN VITRO* ENZYME ACTIVITY[a]

Time after initial exposure (days)	Cd treatment[b] (mg/kg)	I. Dehydrogenation[c] HCCH (μg/mg prot/30 min)	II. Dechlorination[d] TCCH (μg/mg prot/30 min)	III. Hydroxylation[e] PCCOL (μg/mg prot/30 min)	IV. Cytochrome *P*-450 (nmol/mg protein)	Treatment comparison[f]
3	1. 0.00	0.62 ± 0.08	1.12 ± 0.23	7.56 ± 2.78	1.15 ± 0.08	I. 1 > All
	2. 1.04	0.30 ± 0.05	0.35 ± 0.06	2.51 ± 0.79	0.75 ± 0.13	II. 1 > All
	3. 3.20	0.23 ± 0.03	0.16 ± 0.04	1.94 ± 0.65	0.55 ± 0.09	III. N.S.
	4. 0.75	0.28 ± 0.05	0.41 ± 0.12	1.97 ± 0.98	0.91 ± 0.14	IV. 1 > 2,3
7	1. 0.00	0.94 ± 0.04	1.43 ± 0.45	8.48 ± 1.24	1.42 ± 0.29	I. N.S.
	2. 1.04	0.40 ± 0.05	0.35 ± 0.07	4.17 ± 0.23	1.09 ± 0.13	II. N.S.
	3. 3.20	0.33 ± 0.12	0.29 ± 0.07	2.92 ± 0.46	0.85 ± 0.14	III. 1 > All
	4. 0.75	0.44 ± 0.16	1.16 ± 0.43	4.92 ± 1.01	1.40 ± 0.26	IV. N.S.
14	1. 0.00	0.54 ± 0.03	0.92 ± 0.12	7.93 ± 0.79	1.37 ± 0.10	I. 1 > All
	2. 1.04	0.23 ± 0.03	0.21 ± 0.04	3.40 ± 0.37	1.14 ± 0.10	II. 1 > All
	3. 3.20	0.20 ± 0.02	0.16 ± 0.07	3.51 ± 0.24	1.13 ± 0.06	III. 1 > All
	4. 0.75	0.23 ± 0.02	0.37 ± 0.15	3.73 ± 0.19	1.14 ± 0.09	IV. N.S.
21	1. 0.00	0.66 ± 0.13	1.32 ± 0.20	8.56 ± 1.05	1.62 ± 0.07	I. 1 > All
	2. 1.04	0.26 ± 0.07	0.17 ± 0.04	3.62 ± 0.16	1.24 ± 0.12	II. 1 > All; 4 > 2,3
	3. 3.20	0.31 ± 0.06	0.16 ± 0.11	2.87 ± 0.19	1.11 ± 0.05	III. 1 > All
	4. 0.75	0.22 ± 0.08	0.74 ± 0.08	3.40 ± 0.65	1.36 ± 0.11	IV. 1 > 2,3
35	1. 0.00	0.46 ± 0.07	1.33 ± 0.25	12.6 ± 1.74	1.76 ± 0.17	I. 1 > All
	2. 1.04	0.23 ± 0.04	0.44 ± 0.11	6.18 ± 1.23	1.61 ± 0.18	II. 1 > All
	4. 0.75	0.15 ± 0.15	0.32 ± 0.10	4.03 ± 0.64	1.15 ± 0.12	III. 1 > All
						IV. 1 > 4

[a] Values are means ± SE.
[b] Treatment described under Methods.
[c] *In vitro* metabolism of lindane to hexachlorocyclohexene (HCCH).
[d] *In vitro* metabolism of lindane to γ-3,4,5,6-tetrachlorocyclohex-1-ene (TCCH).
[e] *In vitro* metabolism of hexachlorocyclohexene (HCCH) to 2,3,4,5,6-pentachloro-2-cyclohexen-1-ol (PCCOL).
[f] Comparisons are significantly different at $P < 0.05$ by Duncan's Multiple Range Test.

TABLE 3

EFFECT OF CADMIUM ON EXCRETION AND STORAGE OF RADIOACTIVITY[a]

Time after initial exposure (days)	Cd treatment[b] (mg/kg)	I. Total excreted radioactivity		II. Radioactivity in urine		III. Radioactivity in feces		IV. Radioactivity in fat		
		dpm × 10^{-5}	% of Control	dpm × 10^{-5}	% of Control	dpm × 10^{-5}	% of Control	dpm × 10^{-5}/ g lipid	% of Control	Treatment comparisons[c]
3	1. 0.00	18.2 ± 2.21	100	13.6 ± 1.66	100	4.59 ± 0.85	100	1.40 ± 0.07	100	I. 1 > All
	2. 1.04	10.9 ± 0.63	59.9	8.33 ± 0.53	61.2	2.60 ± 0.50	56.6	3.17 ± 0.22	226	II. 1 > All
	3. 3.20	7.78 ± 0.64	42.7	6.38 ± 0.67	46.9	1.40 ± 0.64	30.5	2.99 ± 0.10	214	III. 1 > 3
	4. 0.75	11.1 ± 1.24	61.0	7.76 ± 1.23	57.0	3.35 ± 0.51	73.0	2.09 ± 0.37	149	IV. 2,3 > 1
7	1. 0.00	18.2 ± 2.16	100	13.3 ± 1.48	100	4.92 ± 0.92	100	1.07 ± 0.10	100	I. N.S.
	2. 1.04	14.0 ± 1.79	76.9	10.9 ± 1.31	82.0	3.12 ± 0.54	63.4	2.09 ± 0.12	195	II. N.S.
	3. 3.20	14.4 ± 1.78	79.1	12.0 ± 1.87	90.2	2.49 ± 0.63	50.6	4.01 ± 0.49	375	III. 1 > 3
	4. 0.75	16.2 ± 0.86	89.0	13.0 ± 0.59	97.7	3.16 ± 0.28	64.2	1.58 ± 0.13	148	IV. 3,2 > 1
14	1. 0.00	15.8 ± 0.80	100	11.9 ± 0.64	100	3.86 ± 0.26	100	0.92 ± 0.10	100	I. N.S.
	2. 1.04	13.1 ± 1.00	82.9	10.0 ± 0.96	84.0	3.12 ± 0.18	80.8	1.74 ± 0.02	189	II. N.S.
	3. 3.20	16.2 ± 1.89	102	12.6 ± 1.47	106	3.57 ± 0.57	92.5	2.73 ± 0.52	297	III. 4 > 2
	4. 0.75	15.2 ± 1.35	96.2	11.0 ± 1.21	92.4	4.23 ± 0.17	110	0.96 ± 0.10	104	IV. 3 > 1
21	1. 0.00	15.7 ± 1.39	100	13.1 ± 0.96	100	2.65 ± 0.62	100	1.17 ± 0.04	100	I. 1,4 > 2
	2. 1.04	8.73 ± 1.40	55.6	6.75 ± 1.35	51.5	1.97 ± 0.25	74.3	1.23 ± 0.05	105	II. 1,4 > 2
	3. 3.20	12.1 ± 1.50	77.1	9.84 ± 1.42	75.1	2.28 ± 0.36	86.0	1.66 ± 0.47	142	III. N.S.
	4. 0.75	14.0 ± 2.09	89.2	10.7 ± 0.84	81.7	3.31 ± 1.30	125	1.54 ± 0.31	132	IV. N.S.
35	1. 0.00	21.2 ± 2.33	100	16.8 ± 1.89	100	4.42 ± 0.62	100	0.65 ± 0.12	100	I. 1 > All
	2. 1.04	12.5 ± 2.13	59.0	10.3 ± 1.90	61.3	2.18 ± 0.25	49.3	1.06 ± 0.19	163	II. 1 > All
	4. 0.75	13.2 ± 1.93	62.3	10.6 ± 1.06	63.1	2.57 ± 0.95	58.1	0.87 ± 0.07	134	III. 1 > 2
										IV. N.S.

[a] Values are means ± SE.
[b] Treatment described under Methods.
[c] Comparisons are significantly different at $P < 0.05$ by Duncan's Multiple Range Test.

TABLE 4

EFFECT OF CADMIUM ON PROPORTIONS OF RADIOLABELED URINARY METABOLITES[a]

Time after initial exposure (days)	Cd treatment[b] (mg/kg)	Average percentage of radioactivity excreted in urine extracts				Treatment comparisons[g]
		I. Neutral metabolites[c]	II. Free chlorophenols[d]	III. Conjugated chlorophenols[e]	IV. Polar metabolites[f]	
3	1. 0.00	11.6 ± 1.41	17.4 ± 1.68	45.6 ± 1.34	25.2 ± 0.53	I. 1 > 3,2
	2. 1.04	3.42 ± 0.47	21.0 ± 1.22	43.2 ± 0.34	32.3 ± 0.64	II. N.S.
	3. 3.20	3.50 ± 1.22	19.9 ± 1.86	42.7 ± 2.48	33.8 ± 1.26	III. N.S.
	4. 0.75	7.38 ± 2.28	18.7 ± 0.80	40.4 ± 5.23	33.4 ± 3.73	IV. All > 1
7	1. 0.00	14.1 ± 4.78	17.5 ± 1.35	39.2 ± 4.29	29.0 ± 2.30	I. 1 > 3
	2. 1.04	4.75 ± 0.81	19.4 ± 2.51	40.5 ± 2.62	35.5 ± 1.66	II. N.S.
	3. 3.20	2.47 ± 0.45	18.8 ± 2.04	41.2 ± 1.52	37.5 ± 1.51	III. N.S.
	4. 0.75	11.7 ± 3.11	17.6 ± 1.00	41.5 ± 2.45	29.3 ± 1.45	IV. 3,2 > 4,1
14	1. 0.00	11.8 ± 3.19	17.4 ± 0.45	38.3 ± 2.17	32.6 ± 1.37	I. 4,1 > 3,2
	2. 1.04	4.32 ± 1.24	21.6 ± 1.27	38.8 ± 0.76	35.2 ± 0.79	II. N.S.
	3. 3.20	4.60 ± 1.17	19.4 ± 1.78	37.1 ± 1.11	39.0 ± 0.69	III. N.S.
	4. 0.75	16.0 ± 2.40	17.8 ± 1.30	37.0 ± 0.74	29.1 ± 1.00	IV. 3 > All; 2,1 > 4
21	1. 0.00	24.2 ± 3.16	16.4 ± 1.27	35.2 ± 1.84	24.0 ± 1.12	I. N.S.
	2. 1.04	14.9 ± 5.81	23.7 ± 1.66	31.9 ± 3.20	29.6 ± 1.88	II. 2 > 3,4,1
	3. 3.20	19.3 ± 7.84	18.5 ± 2.26	29.5 ± 3.21	32.9 ± 2.64	III. N.S.
	4. 0.75	24.0 ± 2.60	17.4 ± 1.22	33.0 ± 2.58	25.5 ± 0.69	IV. 3 > 4,1; 2 > 1
35	1. 0.00	14.2 ± 3.40	16.5 ± 0.39	39.5 ± 1.76	29.8 ± 1.82	I. 4,1 > 2
	2. 1.04	3.70 ± 0.60	21.9 ± 1.48	38.2 ± 1.37	36.1 ± 0.91	II. 2 > 1,4
	4. 0.75	18.0 ± 3.26	16.4 ± 0.47	36.0 ± 2.86	29.5 ± 1.09	III. N.S.
						IV. 2 > 1,4

[a] Values are means ± SE.
[b] Treatment described under Methods.
[c] Percentage radioactivity extracted with benzene from acid urine (pH = 2) which does not partition into 0.15 N NaOH.
[d] Percentage radioactivity extracted with benzene from acid urine (pH = 2) which does partition into 0.15 N NaOH.
[e] Percentage radioactivity extracted with benzene from hydrolyzed urine.
[f] Percentage radioactivity in residual urine after extraction.
[g] Comparisons are significantly different at $P < 0.05$ by Duncan's Multiple Range Test.

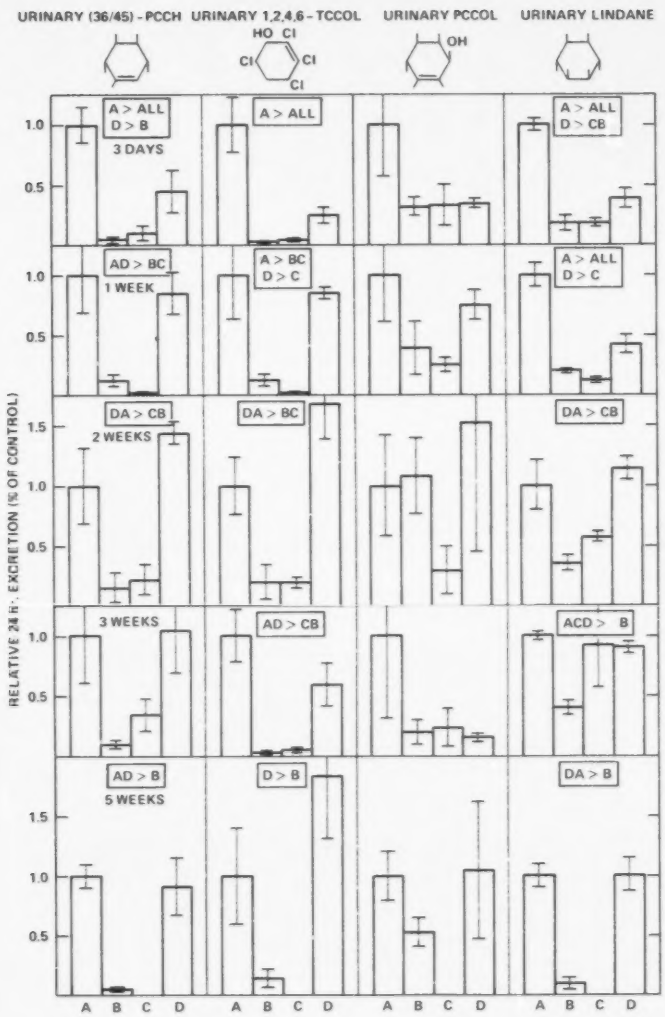

FIG. 1. Effect of acute and chronic Cd treatment on the excretion of neutral lindane metabolites. For each metabolite the mean value of the control bar is represented as 100%. The mean values of the treatment bars relative to that of the controls are presented as percentage ± SE (Cd-treated mean/control mean). The vertical lines indicate the SE. The treatment designations A, B, C, D at the bottom of the figure represent controls, 1.04, 3.20, and 0.75 mg of Cd/kg (three times/week), respectively. Significant comparisons are indicated where $P < 0.05$ by Duncan's Multiple Range Test. Where the configurations of the metabolites are known this is indicated by fractional notation, numbers in the numerator denoting chlorine above and numbers in the denominator denoting chlorine below the plane of the ring. Abbreviations: TCCOL, tetrachlorocyclohexenol; PCCOL, pentachlorocyclohexenol; PCCH, pentachlorocyclohexene.

significantly higher proportion of highly polar metabolites than the controls. The control animals, on the other hand, excreted a significantly higher proportion of neutral metabolites than the rats receiving the acute doses of Cd. This neutral metabolite fraction included γ-2,3,4,5,6-pentachlorocyclohex-1-ene (36/45-PCCH), 2,3,4,6-

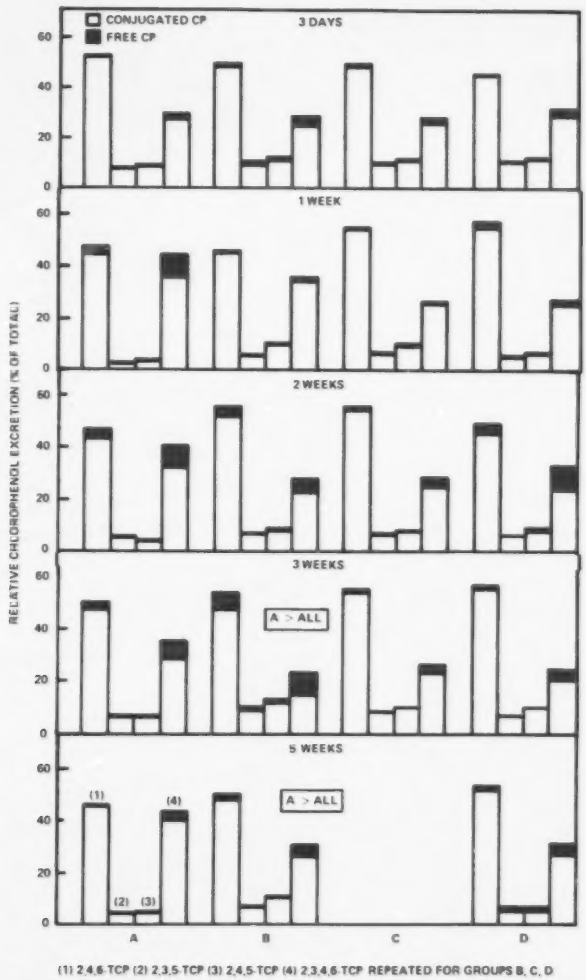

FIG. 2. Effect of acute and chronic Cd treatment on the excretion pattern of free and conjugated chlorophenol metabolites. The excretion patterns (left to right at each sacrifice period) for each treatment group A–D consists of the relative proportions of 2,4,6-, 2,3,5-, 2,4,5-trichlorophenol and 2,3,4,6-tetrachlorophenol from the total chlorophenol excretion. The treatment designations A, B, C, D at the bottom of the figure represent controls, 1.04, 3.20, and 0.75 mg of Cd/kg (three times/week). Significant differences in the 2,3,4,6-tetrachlorophenol/2,4,6-trichlorophenol ratio by Duncan's Multiple Range Test are indicated.

tetrachloro-2-cyclohexen-1-ol (1,2,4,5-TCCOL), 2,3,4,5,6-pentachloro-2-cyclohexen-1-ol (PCCOL), and unaltered lindane (Fig. 1). In this instance the chronic Cd treatment seemed to afford some protection and within a week the proportion of radioactivity in

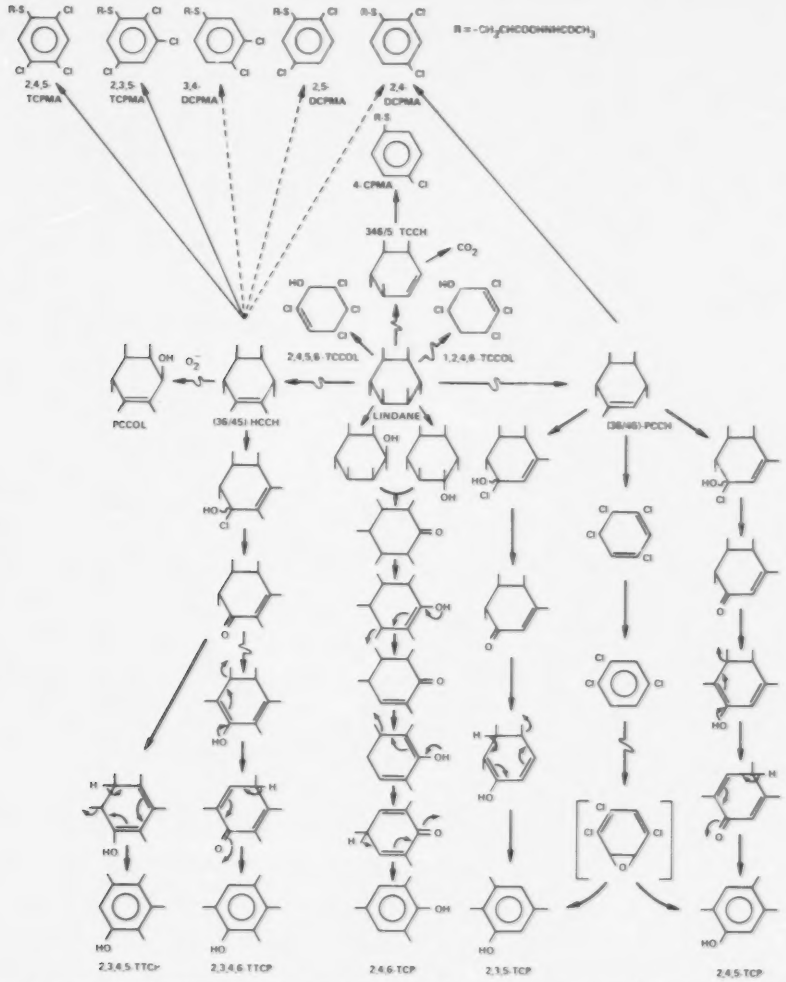

FIG. 3. Effect of Cd treatment on the metabolism of lindane. The wavy arrows indicate the metabolic pathways affected by Cd in this study. Where the configuration of the metabolites is known, this is indicated by fractional notation, numbers in the numerator denoting chlorine above and numbers in the denominator denoting chlorine below the plane of the ring. Abbreviations: TCCH, tetrachlorocyclohexene; TCCOL, tetrachlorocyclohexenol; PCCOL, pentachlorocyclohexenol; HCCH, hexachlorocyclohexene; PCCH, pentachlorocyclohexene; TTCP, tetrachlorophenol; TCP, trichlorophenol; TCPMA, trichlorophenyl mercapturic acid; DCPMA, dichlorophenyl mercapturic acid; CPMA, chlorophenyl mercapturic acid.

TABLE 5

CADMIUM AND ZINC CONTENT OF LIVER AND KIDNEY[a]

Time after initial exposure (days)	Cd treatment[b] (mg/kg)	Liver metal content		Kidney metal content		Treatment comparison[c]
		I. Cadmium (μg/g)	II. Zinc (μg/g)	III. Cadmium (μg/g)	IV. Zinc (μg/g)	
3	1. 0.00	<1	26.0 ± 1.08	<1	18.2 ± 0.63	I. All > 1; 3 > 2,4
	2. 1.04	39.2 ± 2.46	58.0 ± 2.74	14.5 ± 0.87	24.0 ± 0.91	II. All > 1
	3. 3.20	61.5 ± 7.64	53.0 ± 2.71	31.8 ± 4.53	24.0 ± 0.91	III. All > 1; 3 > 2,4
	4. 0.75	32.8 ± 3.38	59.5 ± 4.29	7.50 ± 1.26	21.2 ± 1.11	IV. 3,2 > 1
7	1. 0.00	<1	24.2 ± 1.49	<1	17.8 ± 1.44	I. All > 1; 3 > 2,4
	2. 1.04	40.8 ± 7.46	56.5 ± 5.17	20.0 ± 4.04	28.2 ± 1.25	II. All > 1; 3 > 2,4
	3. 3.20	64.0 ± 7.52	75.5 ± 5.87	63.5 ± 14.7	29.5 ± 1.85	III. All > 1; 3 > 2,4
	4. 0.75	20.5 ± 2.33	44.5 ± 3.01	7.50 ± 1.19	22.5 ± 0.64	IV. All > 1; 3,2 > 4
14	1. 0.00	<1	26.8 ± 0.63	<1	27.2 ± 1.49	I. All > 1; 3 > 2
	2. 1.04	39.7 ± 2.90	54.7 ± 0.67	25.7 ± 0.67	28.0 ± 0.00	II. All > 1
	3. 3.20	58.3 ± 9.17	60.3 ± 5.70	94.0 ± 3.78	33.3 ± 1.76	III. All > 1; 3 > 2,4; 2 > 4
	4. 0.75	42.2 ± 2.63	55.2 ± 2.78	14.5 ± 0.96	26.5 ± 0.29	IV. 3 > All
21	1. 0.00	<1	27.0 ± 1.47	<1	16.5 ± 0.29	I. All > 1; 4 > 2
	2. 1.04	38.8 ± 4.19	56.0 ± 2.86	24.5 ± 2.02	27.0 ± 0.91	II. All > 1
	3. 3.20	56.7 ± 7.06	68.3 ± 9.49	82.7 ± 23.2	27.7 ± 1.76	III. All > 1; 3 > 4,2
	4. 0.75	65.5 ± 7.23	71.8 ± 6.05	29.8 ± 1.70	27.5 ± 1.26	IV. All > 1
35	1. 0.00	<1	25.5 ± 0.64	<1	21.8 ± 3.82	I. All > 1; 4 > 2
	2. 1.04	43.2 ± 1.70	50.0 ± 0.82	36.8 ± 1.75	33.0 ± 6.06	II. All > 1; 4 > 2
	4. 0.75	116 ± 13.7	73.8 ± 3.30	58.0 ± 4.45	45.2 ± 3.35	III. All > 1; 4 > 2
						IV. 4 > 1

[a] Values are means ± SE.
[b] Treatment described under Methods.
[c] Comparisons are significantly different at $P < 0.05$ by Duncan's Multiple Range Test.

the four urinary fractions (Table 4) as well as the amount of the individual neutral metabolites resembled that of the controls.

In addition, though the proportion of radioactivity in the free and conjugated chlorophenol fractions was not significantly different (Table 4), the proportion of the individual chlorophenols was significantly altered by the Cd treatment (Fig. 2). At both 3 weeks and 5 weeks after treatment the ratio of excreted 2,3,4,6-tetrachlorophenol to that of 2,4,6-trichlorophenol was significantly reduced in the rats dosed with Cd. Here repeated Cd administration did not prevent the alteration in metabolism. Lindane is metabolized to 2,3,4,6-tetrachlorophenol by a pathway distinct from that leading to 2,4,6-trichlorophenol (Tanaka *et al.*, 1977; Fig. 3). The two competing pathways respond differently to induction by organochlorine pesticides (Chadwick *et al.*, 1977b), the more sensitive being that which leads to 2,3,4,6-tetrachlorophenol. This study indicates that this pathway is also more sensitive to inhibiton by environmental toxicants such as Cd.

The observed effects of Cd treatment on the metabolism of lindane are summarized in Fig. 3 in which the wavy arrows indicate the metabolic pathways affected. The proposed mechanisms of lindane metabolism are a composite of previous observations and current reports (Grover and Sims, 1965; Chadwick and Freal, 1972; Freal and Chadwick, 1973; Chadwick *et al.*, 1975b; Chadwick *et al.*, 1977b; Tanaka *et al.*, 1977; Kurihara *et al.*, 1977).

The cadmium and zinc content of livers and kidneys from the rats at all five sacrifice periods are presented in Table 5. The hepatic concentration of cadmium in the rats receiving a single dose of 1.04 or 3.20 mg of Cd/kg had reached peak concentration by 3 days and did not change significantly during the rest of the experiment. Livers from the rats repeatedly dosed with 0.75 mg of Cd/kg, on the other hand, showed a cadmium buildup throughout the study. The hepatic zinc concentration in the Cd-treated rats was significantly higher than that of the control animals throughout the study. Livers from rats repeatedly dosed with 0.75 mg of Cd/kg exhibited increasing zinc concentration as Cd accumulated.

In the kidneys there was an increasing accumulation of Cd in all the rats exposed to this heavy metal to the 2 week sacrifice. The rats receiving the chronic Cd treatment continued to show increased Cd accumulation for the duration of the experiment whereas the acutely dosed rats leveled off at 2 weeks. Though not as pronounced or as consistent as the increases observed in the liver, kidney zinc levels were significantly elevated in Cd-treated rats over that of controls.

DISCUSSION

Results from this study indicated that the heavy metal cadmium produced a potent inhibition of lindane metabolism as early as 3 days after treatment and as late as 5 weeks after treatment. Although the Cd-treated rats appeared to be recovering after 1 or 2 weeks, both *in vitro* enzyme activities (Tables 1 and 2) and *in vivo* lindane metabolism (Table 3, Fig. 1) were significantly impaired again 3 and 5 weeks after treatment began. Response of the rats to chronic Cd administration indicated that tolerance to the heavy metal was not evident 5 weeks after treatment began (Tables 1–3, Fig. 1).

Inhibition of the drug-metabolizing enzymes within a week after administration of a single dose of Cd has been widely reported (Unger and Clausen, 1973; Hadley *et al.*, 1974; Tsang and Furst, 1976; Yoshida *et al.*, 1976; Krasny and Holbrook, 1977; Teare *et al.*, 1977; Schnell, 1978). Moreover, a concentration-dependent inhibition of *in vitro* enzyme activity suggested that cadmium could have been acting directly upon the drug-metabolizing enzymes (Hadley *et al.*, 1974; Tsang and Furst, 1976; Schnell, 1978). However, dose-dependent reductions in hepatic cytochrome *P*-450 (Schnell, 1978) and elevated levels of microsomal heme oxygenase (Krasny and Holbrook, 1977; 1978) indicated that Cd may also have inhbited hepatic drug-metabolizing enzymes by indirect mechanisms.

Recovery from Cd-induced inhibition with time has also been previously noted (Hadley *et al.*, 1974; Schnell *et al.*, 1974; Yoshida *et al.*, 1976; Krasny and Holbrook, 1977). However, the recurrence of inhibition at a later time has not been previously reported. Workers who have studied the time dependence of Cd-induced inhibition agreed, in general, that peak potentiation occurred from 2 to 5 days after treatment (Hadley *et al.*, 1974; Schnell *et al.*, 1974). However, neither study investigated Cd-induced inhibition beyond 17 days after treatment.

It has been shown that administration of Cd by either the oral or parenteral route resulted in the accumulation of hepatic Cd which was bound to a specific protein characterized by Kägi and Vallee (1960) and called metallothionein. It has been further demonstrated that the level of Cd in the 30,000*g* liver supernatant, which contains metallothionein, increased to more than 90% of the hepatic Cd content by 6 days after a single subcutaneous dose of 2.5 mg of Cd/kg (Sugawara, 1977). The affinity of metallothionein for Cd is strong and persistent (Nordberg *et al.*, 1972; Chen *et al.*, 1975; Sugawara, 1977). Furthermore, it has been shown that 20 days after a subcutaneous injection of 2.5 mg of Cd/kg, thionein-bound Cd was still increasing (Sugawara, 1977). Thus, it seems unlikely that this heavy metal plays a direct role in the inhibition noted in this experiment, 3 and 5 weeks after treatment.

A possible explanation for the recurrence of inhibition noted in this study could involve long-term Cd-induced alterations in the metabolism and disposition of essential trace elements in the liver. For example, the trace element zinc has been implicated in the potentiation of various hepatic drug-metabolizing enzymes both directly (Peters and Fouts, 1970; Thomson *et al.*, 1974; Tsang and Furst, 1976) and indirectly (Chvapil *et al.*, 1972, 1975). After parenteral administration of Cd, the hepatic content of cadmium thionein has been reported to increase in proportion to the dose and to be accompanied by a concomitant increase in total and thionein-bound Zn (Colucci *et al.*, 1975; Winge *et al.*, 1975). Consistent with these results, Cd treatment in this study produced an elevated hepatic zinc content which remained significantly higher than that of controls throughout the 5 weeks (Table 5). However, it has been reported that while total liver zinc content was maximally elevated 20 days after a subcutaneous injection of 2.5 mg of Cd/kg, the proportion in the 30,000*g* supernatant, which contains metallothionein, declined from that observed 6 days after treatment (Sugawara, 1977). Moreover, the distribution of zinc in proteins from the metallothionein fractions was altered at the 20-day period when compared with that 6 days after Cd treatment. It was concluded that at 20 days zinc had begun to be released from metallothionein while thionein-bound Cd was still increasing (Sugawara, 1977). These observations lead to speculation that the

presence of an increased level of Cd can disturb the endogenous zinc pool so that there is an eventual increase in intracellular free zinc which can, in turn, inhibit metabolism of xenobiotics such as lindane, the organochlorine insecticide used in this study.

Alternatively the high levels of Cd and/or Zn in the liver may adversely affect hepatic Cu content. Copper deficiency is known to potentiate both *in vitro* and *in vivo* metabolism of drugs (Moffitt and Murphy, 1973a,b). Moreover, the administration of cadmium to animals fed diets containing nutritionally adequate levels of Cu has resulted in depressed hepatic copper levels (Campbell and Mills, 1974; Doyle and Pfander, 1975). In addition, the antagonistic action of Zn and Cu metabolism and disposition is well documented (Grant-Frost and Underwood, 1958; Cox and Harris, 1960; Magee and Matrone, 1960; Lee and Matrone, 1969; Whanger and Weswig, 1971; Cox, 1972; Chu and Cox, 1972; Campbell and Mills, 1974).

Thus, it is concluded that a high level of free zinc in the liver and/or a low level of hepatic copper could be responsible for the metabolic inhibition observed in this study 3 and 5 weeks after Cd treatment commenced.

It has been reported that subthreshold doses of Cd (less than 0.84mg/kg ip) administered from 2 weeks to 14 hr prior to a threshold dose prevented the cadmium-induced inhibition of drug metabolism (Schnell, 1978). Nevertheless, chronic Cd treatment did not completely protect the animals in this study from Cd-induced inhibition of the *in vitro* (Tables 1 and 2) or *in vivo* (Table 3, Fig. 1) lindane metabolism 3 and 5 weeks after treatment began. It appears that repeatedly dosing animals sc with subthreshold levels of Cd provided little protection against the inhibition noted 5 weeks after treatment began. Results from this study corroborate a similar observation by Garg and Kourounakis (1976) who reported significant inhibition of ethylmorphine demethylation in male rats dosed daily with 2 mg Cd/kg ip for 49 days.

In summary, acute and chronic Cd treatment produced an early inhibition of lindane metabolism due to the direct and/or indirect action of the heavy metal on the hepatic microsomal enzymes. Then there was a partial recovery in enzyme activity and metabolism 1 to 2 weeks after treatment probably due to the sequestration of Cd by metallothionein. This was followed by the recurrence of significant impairment 3 and 5 weeks after treatment possibly due to secondary effects of cadmium on the metabolism and disposition of the essential trace elements copper and zinc. Chronic Cd exposure provided little protection against the Cd-induced inhibition 5 weeks after treatment commenced.

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